

Sheet 1 of 17 Appl. No. 09/756,283; Filed: Jan 9, 2001 Dkt No. 0623.1000000/LBB/PAJ; Group Unit: 1646 Inventors: Chernajovsky, et al Tel: 202-371-2600 Title: Latent Fusion Protein

TGFb+MMP+ifn b Sequence

10	20	20	40			
10		30	40	50	60	
123456/890	1234567890	123456/890	123456/890	1234567890	1234567890	
				. •		
	CCGGGCTGCG					60
MetProProS	erGlyLeuAr	gLeuLeuPro	LeuLeuLeuP	roLeuLeuTr	pLeuLeuVa1	
CTGACGCCTG	GCCCGCCGGC	CGCGGGACTA	TCCACCTGCA	AGACTATCGA	CATGGAGCTG	120
LeuThrProG	1yProProA1	aAlaGlyLeu	SerThrCvsL	vsThrIleAs	pMetGluLeu	
	•					
GTGAAGCGGA	AGCGCATCGA	GGCCATCCGC	GGCCAGATCC	TGTCCAAGCT	GCGGCTCGCC	180
	ysArgI1eG1					100
vaily shi gl	y shi gi icui	unialienty	diyamireL	euser Lyste	uAI YLEUA I a	
VCCCCCCCV	CCCACCCCCA	CCTCCCCCC	CCCCCCCTCC	CCCACCCCCT	CCTCCCCCTC	0.40
	GCCAGGGGA					240
SerProProS	erGlnGlyGl	uvalProPro	GlyProLeuP	roGluAlaVa	ILeuAIaLeu	
	CCCGCGACCG					300
TyrAsnSerT	hrArgAspAr	gValAlaGly	GluSerAlaG	luProGluPr	oGluProGlu	
GCCGACTACT	ACGCCAAGGA	GGTCACCCGC	GTGCTAATGG	TGGAAACCCA	CAACGAAATC	360
AlaAspTyrT	yrA1aLysG1	uValThrArg	ValLeuMetV	alGluThrHi	sAsnGluIle	
, ,						
TATGACAAGT	TCAAGCAGAG	TACACACAGC	ATATATATGT	TCTTCAACAC	ATCAGAGCTC	420
	heLysG1nSe					0
1317(SPL) 31	nelysamoe	1 11111113361	Ticiyilica	net neasitin	1 SCI GIULCU	
CCVCVVCCCC	TACCTGAACC	CCTCTTCCTC	TCCCGGCAG	ACCTCCCTCT	CCTCACCACC	480
						400
Arguiuaiav	alProGluPr	ovaileuleu	Serargalad	TuleuArgle	uLeuArgarg	
0704407744	A A O TO O A O O A	0040070040	0.707400404		0447700700	= 40
	AAGTGGAGCA					540
LeuLysLeuL	ysValGluG1	nHisValGlu	LeuTyrGlnL	ysTyrSerAs	nAsnSerTrp	
CGATACCTCA	GCAACCGGCT	GCTGGCACCC	AGCGACTCGC	CAGAGTGGTT	ATCTTTTGAT	600
ArgTyrLeuS	erAsnArgLe	uLeuAlaPro	SerAspSerP	roGluTrpLe	uSerPheAsp	
	•		·	·	·	
GTCACCGGAG	TTGTGCGGCA	GTGGTTGAGC	CGTGGAGGG	AAATTGAGGG	CTTTCGCCTT	660
	alValArgG1					
	gai	predoci	garjarja	. az i cai aai	J. Horn geod	
ACCCCCC ACT	GCTCCTGTGA	CACCACCCAT	ΛΛΟΛΟΛΟΤΟΟ	AAGTGGACAT	CAACGGGTTC	720
						120
SerATamist	ysSerCysAs	pserargasp	ASHITTLEUG	invaiAspil	easnutyrne	



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ACTACCGGCC GCCGAGGTGA ThrThrGlyA rgArgGlyAs	 	 	780
CTCATGGCCA CCCCGCTGGA LeuMetAlaT hrProLeuGl			840
TCCCCGCTCG GGCTTTGGGC SerProLeuG lyLeuTrpAl			900
CAGCTCCAAG AAAGGACGAA GlnLeuGlnG luArgThrAs	 	 	960
AAGATCAACC TCACCTACAG LysIleAsnL euThrTyrAr	 	 	1020
CAGAAGAGTT ACACTGCCTT GlnLysSerT yrThrAlaPh			1080
AGAAACAATT TCTCCAGCAC ArgAsnAsnP heSerSerTh	 	 	1140
CTCCACCAGC AGACAGTGTT LeuHisGlnG lnThrValPh			1200
ACGTGGGAGA TGTCCTCAAC ThrTrpGluM etSerSerTh	 	 	1260
TACCTTAAAC TCATGAAGTA TyrLeuLysL euMetLysTy	 	 	1320
AGGAACTTTC TCATCATTCG ArgAsnPheL euIleIleAr	 	 	1376

FIG.1B



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ifn+MMP+TGFb Sequence

				•		
10	20	30	40	50	60	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
ATCA ACA ACA	CCTCCATCCT	CCACGCTGCG	TTCCTCCTCT	CCTTCTCCAC	CACACCCCTC	C 0
		uHisAlaAla				60
MECASIASIA	rgirpriece	UITISATAATA	rneLeuLeuC	ysrneserin	rinimiateu	
TCCATCAACT	ATAAGCAGCT	CCAGCTCCAA	GAAAGGACGA	ACATTCGGAA	ATGTCAGGAG	120
		uGlnLeuGln				
		AAAGATCAAC				180
LeuLeuG1uG	1nLeuAsnG1	yLysIleAsn	LeuThrTyrA	rgAlaAspPh	eLysIlePro	
ATCCACATCA	CCCACAACAT	CCACAACACT	TACACTCCCT	TTCCCATCCA	ACACATCCTC	040
		GCAGAAGAGT tGlnLysSer				240
metarumetr	ili di ulysne	tu iiiLyssei	TyriiiAtar	neAtailedi	narumetteu	
CAGAATGTCT	TTCTTGTCTT	CAGAAACAAT	TTCTCCAGCA	CTGGGTGGAR	TGAGACTATT	300
	*	eArgAsnAsn				,
				•		
		ACTCCACCAG				360
ValValArgL	euLeuAspG1	uLeuHisG1n	GlnThrValP	heLeuLysTh	rValLeuGlu	
GAAAAGCAAG	ACCA A ACATT	GACGTGGGAG	ATCTCCTCAA	CTCCTCTCCA	CTTCAACACC	420
		uThrTrpGlu				420
a rue y su ma	ruarum gee	arm ri para	11000010011	maracam	3 TeuLy 33eT	
TATTACTGGA	GGGTGCAAAG	GTACCTTAAA	CTCATGAAGT	ACAACAGCTA	CGCCTGGATG	480
TyrTyrTrpA	rgValGlnAr	gTyrLeuLys	LeuMetLysT	yrAsnSerTy	rAlaTrpMet	
		CAGGAACTTT				540
ValValArgA	laGlullePh	eArgAsnPhe	LeullelleA	rgArgLeulh	rArgAsnPhe	
CAAAACGAAT	TOGGGGAGG	CGGATCCCCG	CTCGGGCTTT	CCCCCCCVCC	GGGCTCAGCG	600
		yGlySerPro		W 70		000
ammonara	near y ar y ar	Janjoenno	Leadificat	priradiyar	Jaryoerma	
GCCGCACTAT	CCACCTGCAA	GACTATCGAC	ATGGAGCTGG	TGAAGCGGAA	GCGCATCGAG	660
AlaAlaLeuS	${\tt erThrCysLy}$	sThrIleASp	MetG1uLeuV	alLysArgLy	sArgIleGlu	
		GTCCAAGCTG				720
AlalleArgG	lyGinlieLe	uSerLysLeu	ArgLeuSTaS	erProProSe	rGInGlyGlu	



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GTGCCGCCCG GCCCGCTGCC CGAGGCCGTG CTCGCCCTGT ACAACAGCAC CCGCGACCGG ValProProG lyProLeuPr OGluAlaVal LeuAlaLeuT yrASnSerTh rArgAspArg	780
GTGGCCGGGG AGAGTGCAGA ACCGGAGCCC GAGCCTGAGG CCGACTACTA CGCCAAGGAG ValAlaGlyG luSerAlaGl uProGluPro GluProGluA laAspTyrTy rAlaLysGlu	840
GTCACCCGCG TGCTAATGGT GGAAACCCAC AACGAAATCT ATGACAAGTT CAAGCAGAGT ValThrArgV alLeuMetVa lGluThrHis AsnGluIleT yrAspLysPh eLysGlnSer	900
ACACACAGCA TATATATGTT CTTCAACACA TCAGAGCTCC GAGAAGCGGT ACCTGAACCC ThrHisSerl leTyrMetPh ePheAsnThr SerGluLeuA rgGluAlaVa lProGluPro	960
GTGTTGCTCT CCCGGGCACA GCTGCGTCTG CTGAGGAGGC TCAAGTTAAA AGTGGAGCAG ValLeuLeuS erArgAlaGl uLeuArgLeu LeuArgArgL euhysLeuLy sValGluGln	1020
CACGTGGAGC TGTACCAGAA ATACAGCAAC AATTCCTGGC GATACCTCAG CAACCGGCTG HisValGluL euTyrGlnLy sTyrSerAsn AsnSerTrPA rgTyrheuSe rAsnArgLeu	1080
CTGGCACCCA GCGACTCGCC AGAGTGGTTA TCTTTTGATG TCACCGGAGT TGTGCGGCAG LeuAlaProS erAspSerPr oGluTrpLeu SerPheAspV alThrGlyVa lValArgGln	1140
TGGTTGAGCC GTGGAGGGA AATTGAGGGC TTTCGCCTTA GCGCCCACTG CTCCTGTGAC TrpLeuSerA rgGlyGlyGl uIleGluGly PheArgheuS erAlaHisCy sSerCysAsp	1200
AGCAGGGATA ACACACTGCA AGTGGACATC AACGGGTTCA CTACCGGCCG CCGAGGTGAC SerArgAspA snThrLeuGl nValAspIle AsnGlyPheT hrThrGlyAr GArgGlyAsp	1260
CTGGCCACCA TTCATGGCAT GAACCGGCCT TTCCTGCTTC TCATGGCCAC CCCGCTGGAG LeuAlaThrI leHisGlyMe tAsnArgPro PheheuLeuL euMetAlaTh rProLeuGlu	1320
AGGGCCCAGC ATCTGCAAAG GtgaTCTAGA CC ArgAlaGlnH isLeuGlnSe rSerArg	1352

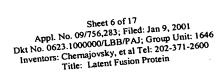
FIG.2B



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1 20 40 60	MPPSGLRLLPLLLPLLWLLV-LTPGPPAAGLSTCKTIOMELVKRKRIEAIRGQILSKLRLASPPSQGE-VP-PGP MHYCVLSAFLILH LVTVALSLSTCSTLDMOQFMRKRIEAIRGQILSKLKLTSPPEDYPEPEE		MEVLWMLLVLLV-LHLSSLANSLSTCKAVDMEEVRKRRIEAIRGQILSKLKLDKIPDVDS-EK-MTV	+ + +++++++++++++++++++++++++++++++++++	80 100 120	LPEAVLALY <u>NST</u> RDRVAGESAEPE-PEPEADYYAKEVIRVLMVETHNEIYDKFKQSTHS <u>JY</u> MFF		VPYQVLALY <u>NST</u> RELLEEHGER-KEEGCTQENTESEYYAKEIHKFDMIQGLAE-HNELAVCPKGIT-SKVFRF	GSPW-RPP-GTAPWSIGPKSCGGSPW-RPP-GTAPWSIG-SRRA	PSEAIF-LYNSTLE-VIREKATRE-EEEHVGHDONIQDYYAKQVYRFESITELEDHEFKFKF	++++	140 160 180			<u>NVSSVEKNRT</u> NLF-RAEFRVLRVP <u>NPS</u> -SKRNEQRIELFQILRP-DEHIAKQRYIGGKNLPTRGTAE			+ + + + + + + +
	3 2 2						3								3			
	Hu TGF-β Hu TGF-β						Hu TGF-			Fg TGF- β			757	75	Hu TGF- β	쥰	75	

FIG.3A





240	WLSFDVTGVVRQWLSRGGEIEGFRLSAHCSGDSRDNTLQVDIN-GFTTGRRGDLATI	TDTVREWLLRRESNLGLEISIHCPCHTFQP.NGDILENIHEVMEIKFK.GVDNEDDHGRGDLGRLKK	WLSFDVTDAVHQWLSGSELIGVFKLSVHCPCEMGPG-HADEMRISIEGFEOQRGDMQSIAK WNSFDVTKTVNEWLKRAEENEQFGLQPAGKGPTPQAKDIDIEGFPAL-RGDLASLSSK	**	IYCFSST EKNCCVRQLY	PHLLLMLLPSYRL-ESQQTNRRKKRALDAAYCFRNVQDNCCLRPLYIDFKRDLGWKWIHEP -N-PHI II MMIPPHRI -DNPGOGGORKKRAI DTNYCFRNIFFNCCVRPI YIDFRODI GWKWVHFP	V-PYVLAMALPAERANELHSARRRROLDTDYCFGPGTDEKNCCVRPLYIDFRKDLQWKWIHEP	KPYLMITSMPAERIDTVT SSRKKRGVGQEYCFGNN-GPNCCVKPLYINFRKDLGWKWIHEP	+++ +++ ++ + ++++ + +++ + + + + + + +	360 380 390	KGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS	-CAGACPYLWSSDTQHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGKIPKIEQLSNMIVKSCKCS	KGYYANFCSGPCPYLKSADTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTTLYYVGRIPKVEQLSNMVVKSCKCS	KGYMANFUMGPUPYIWSADIQYIKVLALYNQHNPGASAAPUUVPQILDPLPIIYYVGRIAKVEQLSNMVVRAUKUS KGYFANYCIGNOPYIWSMDTOVSKVISIYNOHNPGASISPOVPOVIEPIPIIYYVGRIAKVEDISNMVVRSONCS	++++++++++++++++++++++++++++++++++++++
200 220	WLSFDVTGVVRQWLSRGGEIEGFRLSAHC WLSFDVTDAVHEWLHHKDRWLGFKISLHC	WLSFDVTDTVREWLLRRESNLGLEISIHC	-SFDVIDAVHQWLSGSELIGVFKLSVHC NSFDVTKTVNEWLKRAEENEQFGLQPAG	++++++++	HGMWRPFLLLMATPLERA-QKLC	KNSGKT PHLLLMLLPSYRL - ESQ OKDNN N - PHI II MMIPPHRI - DNPGOO		ENTKPYLMITSMPAERIDTVT	+	320 340	3YHANFCLGPCPY IWSLDTQYSKVLALY	KGYNANFCAGACPYLWSSDTQHSRVLSLY	3YYANFCSGPCPYLRSADITHSTVLGLY	KGYMANFCMGPCPY IWSADIQY IKVLALY KGYEANYCI GNCPY IWSMDTQYSKVI SI Y	+++ ++ ++ ++++++++++++++++++++++++++++
			4 C	+	·) 							4 ռ Տ Ճ	•
	Hu TGF-β Hu TGF-β			·	Hu TGF-β	Hu TGF-β Hı TGF-β	-					Hu TGF-β		CK 191-15 Fr 191-15	<u> </u>

FIG.3E



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84 84 79 84 84 85 86 88 88 88 88 88

Reference Sequence Protein

80 80 80 84 84 84

Gly-Pro-Leu-Gly775~Ile776-Ala-Gly-Ile

Gly-Pro-Glu-Gly₆₇₉~Leu₆₈₀-Arg-Val-Gly

Ala-Ala-Tyr-His₆₈₁~Leu₆₈₂-Val-Ser-Gin

Met-Asp-Ala-Phe₆₉₁~Leu₆₉₂-Glu-Ser-Ser Glu-Pro-Gln-Ala₆₈₃~Leu₆₈₄-Ala-Met-Ser

Gly-Pro-Gln-Gly₇₇₅~Leu₇₇₆-Ala-Gly-Gln

Ala-Pro-Gln-Gly775~Ile776-Ala-Gly-Gln Gly-Pro-Gln-Gly775~Leu776-Leu-Gly-Ala 84

Human pregnancy zone protein Human pregnancy zone protein pregnancy zone protein [collagen $(\alpha]$) I collagen (α 2) Human insulin-like growth Human type III collagen type II collagen Human α₂-macroglobulin $lpha_{\scriptscriptstyle 1}$ -protease inhibitor Rat α₂-macroglobulin Aat α,-macroglobulin Human cartilage link dat α,-macroglobulin Rat α,-macroglobulin Chicken ovostatin Human aggrecan Human aggrecan type] Human type MMP-1/MMP-8 Human . Human Human

Gln-Ala-Leu-Ala₆₈₅~Met₆₈₆-Ser-Ala-Ile

Pro-Ser-Tyr-Phe673~Leu674-Asn-Ala-Gly

Tyr-Glu-Ala-Gly685~Leu686-Gly-Val-Va

Ala-Gly-Leu-Gly687~Val688-Val-Glu-Arg Ala-Gly-Leu-Gly757~Ile758-Ser-Ser-Thr

_eu-Arg-Ala-Tyr99 ~Leu100-Leu-Pro-Ala

factor binding protein-3

Arg-Ala-Ile-His₁₆ ~ILe₁₇ -Gln-Ala-Glu

Thr-Glu-Gly-Glu₃₇₃~Ala₃₇₄-Arg-Gly-Ser

[]e-Pro-G]u-Asn341~Phe342-Phe-G]y-Va Gly-Ala-Met-Phe₃₅₂~Leu₃₅₃-Glu-Ala-Il



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Gly-Ala-Hyp-Gly ₅₄₇ ~ Leu ₅₄₈ -Glx-Gly-His Gly-Pro-Gln-G1y ₁₉₀ ~ Val ₁₉₁ -Arg-Gly-Glu Gly-Pro-Ala-Gly ₂₇₇ ~ Val ₂₇₈ -Gln-Gly-Pro Gly-Pro-Ala-Gly ₃₀₁ ~ Leu ₃₀₂ -Hyp-Gly-Pro Gly-Pro-Ala-Gly ₃₃₁ ~ Glu ₃₃₂ - Arg-Gly-Ser Gly-Pro-Ala-Gly ₃₆₂ ~ Gln ₃₈₃ - Asp-Gly-Pro Gly-Pro-Hyl-Gly ₆₇₆ ~ Asn ₆₇₇ - Val-Gly-Ala Gly-Pro-Gln-Gly ₇₇₅ ~ Ile ₇₇₆ - Ala-Gly-Ala Gly-Pro-Glu-Asn ₃₄₁ ~ Phe ₃₄₂ - Phe-Gly-Ala Ile-Pro-Glu-Asn ₃₄₁ ~ Phe ₃₄₂ - Phe-Gly-Val Pro-Pro-Gly-Ala ₆₂ ~ Tyr ₆₃ - His-Gly-Ala Arg-Ala-Ile-His ₁₆ ~ Ile ₁₇ - Gln-Ala-Glu Gly-Pro-His-Leu ₂₅ ~ Leu ₂₆ - Val-Glu-Ala	Gly-Ala-Hyp-Gly ₅₄₇ ~Leu ₅₄₈ -Glx-Gly-His
---	---

gelatin

gelatin

Human insulin-like growth factor

Human cartilage link Human cartilage link

Human galectin-3 Human aggrecan

Bovine 1



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79,92

Human α_2 -macroglobulin Himan α_2 -macroglobulin	Gly-Pro-Glu-Gly679~Leu680-Arg-Val-Gly
	den 19. un 9. j. 1889 - 19. grand and de la company de la
Human $lpha_1$ -antichymotrypsin	Leu-Leu-Ser-Ala ₃₆₀ ~Leu ₃₆₁ -Val-Glu-Thr
$lpha_1$ -protease inhibitor	Glu-Ala-Ile-Pro357~Met358-Ser-Ile-Pro
Antithrombin III	Ile-Ala-Gly-Arg ₃₈₅ ~Ser ₃₈₆ -Leu-Asn-Pro
Chicken ovostatin	Leu-Asn-Ala-Gly ₆₇₇ ~Phe ₆₇₈ -Thr-Ala-Ser
Human aggrecan	Ile-Pro-Glu-Asn ₃₄₁ ~Phe ₃₄₂ -Phe-Gly-Val
Substance P	Lys-Pro-Gln-Gln6 ~Phe7 -Phe-Gly-Leu
Human ProMMP-1	Asp-Val-Ala-Glng0 ~Pheg1 -Val-Leu-Thr
Human ProMMP-3	Asp-Thr-Leu-Glugs ~Valg -Met-Arg-Lys
Human ProMMP3	Asp-Val-Gly-Hisg2 ~Pheg3 -Arg-Thr-Phe
Human ProMMP-8	Asp-Ser-Gly-Gly78 ~Phe79 -Met-Leu-Thr
Human ProMMP-9	Arg-Val-Ala-Glu ₄₀ ~Met ₄₁ -Arg-Gly-Glu
Human ProMMP-9	Asp-Leu-Gly-Args7 ~Phegg -Gln-Thr-Phe
Human fibronectin	Pro-Phe-Ser-Progg~Leuggn-Val-Ala-Thr
Human insulin-like growth factor	Leu-Arg-Ala-Tyrgg ~Leu100-Leu-Pro-Ala
binding protein-3	Ala-Pro-Gly-Asn ₁₀₀ ~Ala ₁₁₀ -Ser-Glu-Ser
	Dha. Sar. Sar. Glii ~ Sar 1 vs. Ard. Glii

Sovine lpha 1 (II) = 1

lpha 1 (II)collagen,N-telope

al(IX)collagen,NC2 a2(IX)collagen,NC2

Bovine Bovine Bovine Bovine

Bovine

al(XI)collagen,N-telopep

Human cartilage link

insulin,B chain insulin,B chain

Bovine

Bovine

a3(IX)collagen,NC2

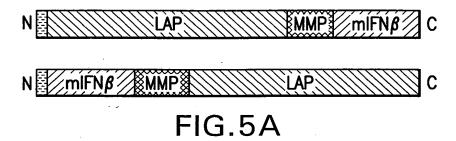


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-IG.4D



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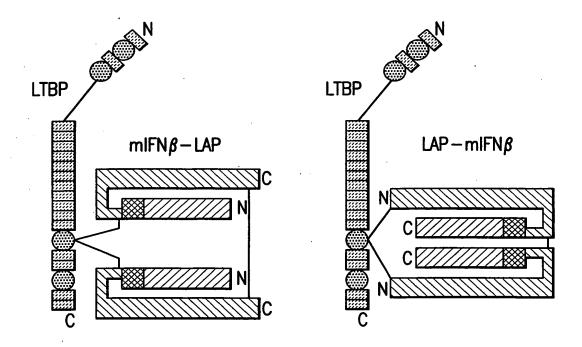


FIG.5B



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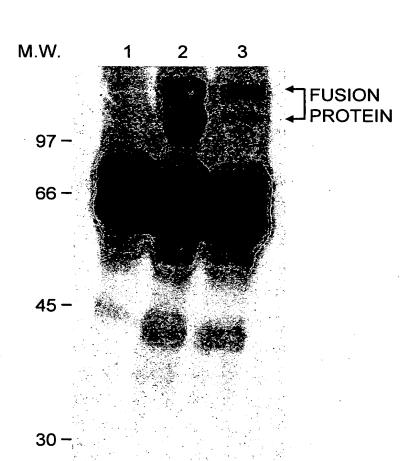


FIG.6



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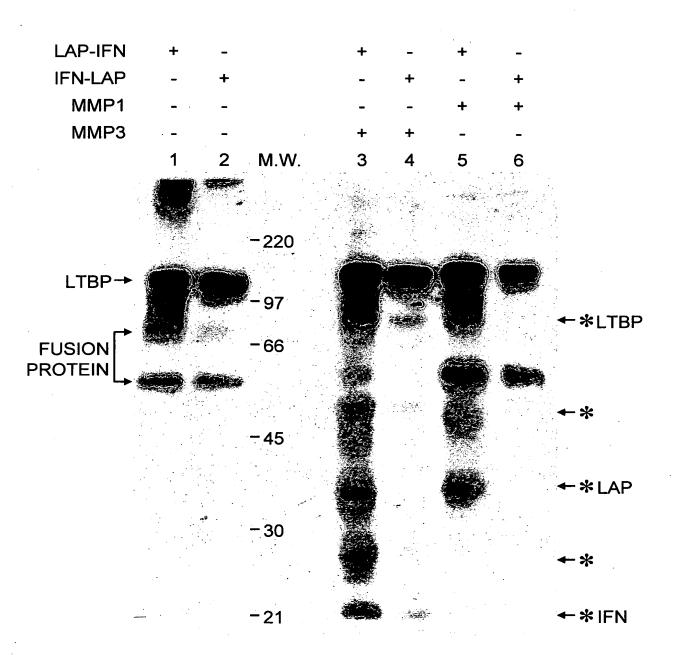


FIG.7



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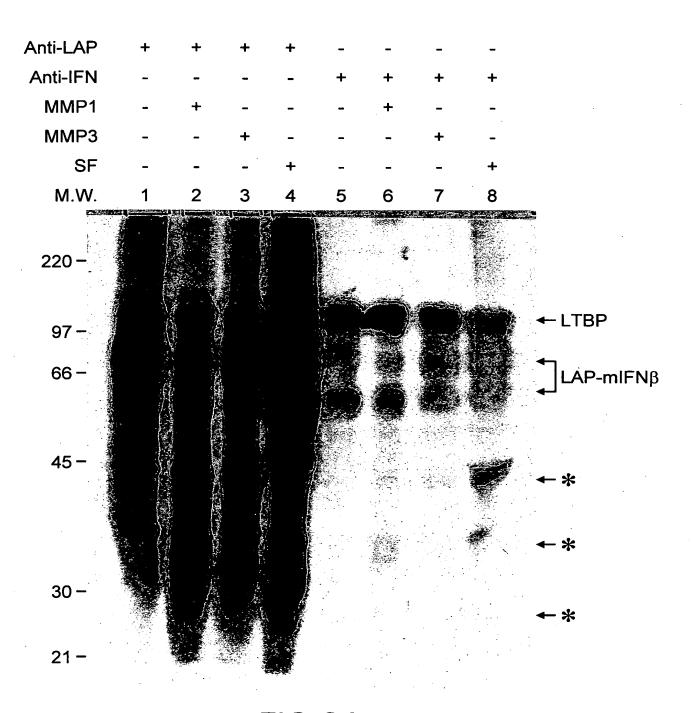


FIG.8A



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Dkt No. 0623.1000000/LBB/PAJ; Group Unit: 1646
Inventors: Chernajovsky, et al Tel: 202-371-2600
Title: Latent Fusion Protein

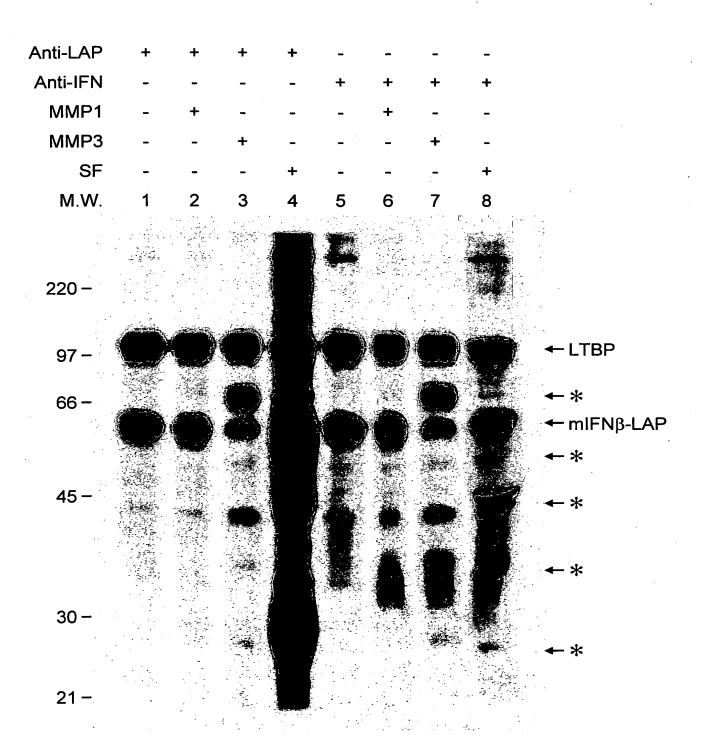
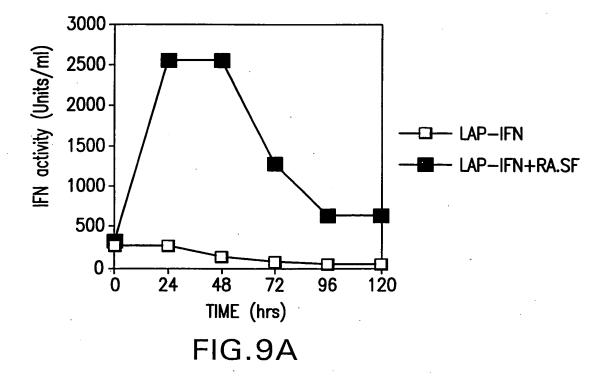
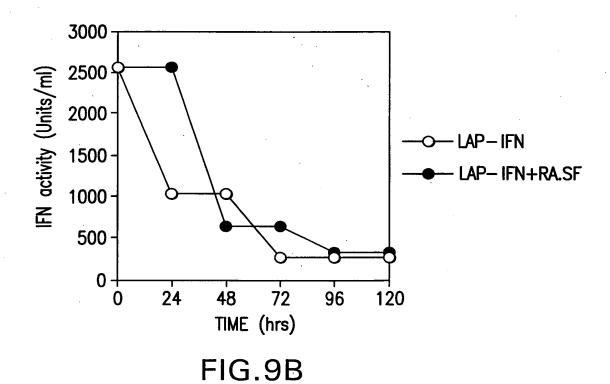


FIG.8B



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